

SHORT PAPER

Does 'affinity' hold the key to fertility in the female mule?

By ANN C. CHANDLEY,

*MRC Clinical and Population Cytogenetics Unit,
Western General Hospital, Edinburgh EH4 2XU, Scotland*

(Received 26 June 1980)

SUMMARY

Michie's hypothesis of 'affinity' is invoked to explain how fertility might theoretically be achieved in a female mule or hinny. Chromosomally balanced haploid gametes could be produced in these inter-specific hybrids by movement of centromeres of similar ancestry to opposite poles at anaphase I of meiosis. Male hybrids could probably never be fertile on account of the low numbers of spermatozoa produced; females, on the other hand, might occasionally achieve fertility by the ovulation of a chromosomally balanced egg.

The sterility of mules (mare \times jack donkey) and hinnies (jenny donkey \times stallion) has been recognized since the time of Aristotle, but rare reports of fertile female mules and hinnies have continued to appear in the scientific literature over the years (Anderson, 1939; Benirschke & Sullivan, 1966; Benirschke *et al.* 1964; Bielanski, 1955, 1972; Ewart, 1899; Gray, 1954; Lloyd-Jones, 1916; Savory, 1970; Smith, 1939). Bielanski (1955), in reviewing all available literature on the subject since 1527, has recorded altogether about 30 cases of alleged fertility in she-mules and 2 or 3 cases in she-hinnies. Fertile male hybrids have, however, never been reported.

Despite a chain of direct and circumstantial evidence, the majority of scientists and even mule breeders themselves are inclined to believe generally that all cases are simply 'errors of observation and recording'. Instances of fertility among female mules and hinnies are few and inadequately documented and in at least one case (Benirschke *et al.* 1964), an allegedly fertile she-mule turned out to be a normal donkey on chromosome analysis.

One apparently well-documented fertile she-mule of the past appears to be 'Old Bec', owned by Texas A. and M. College during the 1920s (Anderson, 1939). Judging from a published photograph she appears to be a typical mule (L. Travis, personal communication), yet she produced two offspring. One was a typical she-mule sired by a donkey, who unlike her mother proved to be sterile, the other was a horse, described as being 'of good conformation', sired by a stallion. This son of 'Old Bec' was not only fertile, producing a number of colts and fillies, but also served as a popular saddle horse around the area. None of his offspring showed any reversion to their donkey ancestry and this absence of ass-like characters led Anderson (1939) to conclude that 'this son of "Old Bec" had originated from an ovum with no chromosomes from his grandsire, the jack'.

The types of progeny produced by 'Old Bec' are in fact consistent with all other reported cases from allegedly fertile mules (Bielanski, 1972; Savory, 1970) in showing that when a female mule is sired by a donkey, the progeny are always mule or 'mule-like' and when sired by a horse, they are typical horse. Furthermore, the progeny of rare fertile

female hinnies, when sired by a donkey, are always donkey (Bielanski, 1955; Savory, 1970). (Matings of female hinnies to stallions appear not to have produced any outcome, but reports of such matings are extremely scarce). There is in fact such a consistency in the reporting of the progeny of these matings that one is led to the same conclusion as Anderson (1939) that the ova of she-mules which produce viable pregnancies carry only horse chromosomes, while those of the hinny carry only donkey chromosomes. In other words, viable pregnancies appear to arise from ova carrying only *maternal* chromosome sets; those carrying paternal chromosomes, or the foetuses resulting from them, appear to be eliminated.

The main impediment to fertility in equine hybrids like the mule and hinny is the breakdown of development in the vast majority of germ-cells during gametogenesis, both in males (Chandley *et al.* 1974; Makino, 1955; Trujillo *et al.* 1969; Wodsedalek, 1916) and females (Taylor & Short, 1973). Synaptic irregularities are a usual feature of primary spermatocytes examined cytologically at meiotic prophase in male hybrids. The difficulties arise, not just because the horse (*Equus caballus*, $2n = 64$) contributes 32 chromosomes to the hybrid and the donkey (*Equus asinus*, $2n = 62$) only 31, but also because structural chromosome differences have developed over evolutionary time (Hsu & Benirschke, 1969), which lead to irregularities when pairing occurs between homologous elements in the horse and donkey sets. Why incomplete meiotic pairing should be associated with gametogenic breakdown is not yet fully understood. However, the general phenomenon has been observed in males from a number of different species and there are several interesting hypotheses on the subject (Miklos, 1974).

In spite of these synaptic difficulties, however, the remarkable thing is that a small number of spermatocytes in male hybrids appear to be able to achieve near-normal pairing at pachytene (Chandley *et al.* 1974). Some reach anaphase I of meiosis (Wodsedalek, 1916) and post-meiotic development into mature spermatozoa has been reported in mules (Bratanov, Dikov, Dokov, 1964), hinnies (Chandley *et al.* 1974; Trujillo *et al.* 1969) and zebra-horse hybrids (Ewart, 1910). Furthermore, when measurements of size and/or DNA content have been made on spermatozoa of mules (Bratanov *et al.* 1964) and hinnies (Chandley *et al.* 1974; Trujillo *et al.* 1969) they have suggested haploidy or quasi-haploidy, rather than the diploidy postulated by some authors (Trujillo *et al.* 1969). In morphology, hinny spermatozoa appear well formed with head, mid-piece and tail (Trujillo *et al.* 1969), although they may be slightly smaller and more variable in size than those of the horse and donkey (Chandley *et al.* 1974). They also appear to be non-motile (Trujillo *et al.* 1969). In female mules and hinnies some germ cells are capable of developing into oocytes (Taylor & Short, 1973) that can even survive to the time of ovulation (Bielanski & Zapletal, 1968; C. E. Adams, W. R. Allen, A. C. Chandley & R. V. Short, unpublished observations), and these few surviving oocytes appear to induce the development of normal follicular cells (Taylor & Short, 1973). The ovary is capable of acquiring some endocrine activity in later life (Taylor & Short, 1973) and it is known that she-mules, hinnies and zebra-horse hybrids may come into oestrus at irregular intervals (Bielanski, 1972; Ewart, 1899; Nishikawa & Sugie, 1952).

The finding of mature gametes in mules and hinnies has not yet been adequately explained in genetic terms. The theoretical considerations of how it might be achieved have, however, been discussed by a number of authors (Anderson, 1939; Chandley *et al.* 1974; Trujillo *et al.* 1969). One possible explanation, originally suggested by Anderson (1939), is that a whole set of donkey chromosomes might occasionally 'cling together' and pass to one pole of the first meiotic spindle, while a whole horse set passed to the other pole. This was a deduction arrived at from consideration of the types of offspring produced by allegedly fertile female mules, but it could also readily explain the finding of haploidy and quasi-haploidy rather than diploidy among the spermatozoa of male hybrids (Chandley *et al.* 1974; Trujillo *et al.* 1969). However, no precise genetic mech-

anism to account for such anomalous segregation has been proposed, and the seemingly improbable odds (1 in 2^{32}) that it could ever happen by chance led Trujillo *et al.* (1969) to reject the idea completely.

A precise mechanism was described some time ago in the genetics literature that might explain the facts. Michie (1953, 1955) proposed a simple hypothesis to explain the anomalous segregation ratios that had been obtained by earlier workers in making distant crosses of mice. The main body of data considered by Michie (1953, 1955) resulted from a cross performed by Gates (1926) between a male 'Japanese' waltzer (a domesticated variety of *Mus bactrianus*) and a female European laboratory mouse (*Mus musculus*). The phenotypic frequencies in backcross progeny from the F_1 hybrids departed strikingly from Mendelian expectations and Gates (1926) drew attention to the marked excess of the *bactrianus* combination of characters. As an interpretation, he postulated an 'association system' – a definite tendency on the part of chromosomes to associate together according to parental grouping, thus distorting the theoretical proportions and resulting in apparent linkages between unlinked loci. Gates's (1926) 'association system' suggested to Michie (1953, 1955) a hypothesis that he believed could explain the observations if it were assumed that at the first meiotic division in the hybrids, *musculus* centromeres tended to pass to one pole and *bactrianus* centromeres to the other. The term 'affinity' was suggested by Fisher to describe the new phenomenon. The belief is (Michie, 1953, 1955) that 'affinity' results from a physical attraction of centromeres of similar ancestry, the centromeres derived from the same parental group having something in common as a basis for their attraction, either for some polar element of the cell or for each other. Data from the mouse hybrids render the latter the more probable alternative (Michie, 1955). 'Affinity' could also explain the observed rapid reversion to parental type in interspecific hybrids (Wallace & Gunn, 1965) and may be of evolutionary significance in maintaining species integrity (Michie, 1955). As a phenomenon, it has also been observed to operate within crosses of laboratory stocks of *Mus musculus* (Wallace, 1953).

The question is, could 'affinity' hold the key to fertility in the mule and hinny? Is it possible that in equine hybrids, with their divergent parental karyotypes, segregation of complete or near-complete maternal or paternal chromosome sets to opposite poles at anaphase I, according to the principles of 'affinity', could occur in the rare germ-cells which survive the meiotic prophase? Could this be the 'clinging together' of whole parental sets of chromosomes that Anderson postulated in 1939? This author believes it might. The observation that mule and hinny spermatozoa are haploid or quasi-haploid rather than diploid would certainly be compatible with a mechanism that drove complete or near-complete whole parental sets of chromosomes to opposite poles at meiosis. The low sperm counts recorded in male mules and hinnies (Bratanov *et al.* 1964; Chandley *et al.* 1974; Trujillo *et al.* 1969) and the apparent lack of motility in those few so produced (Trujillo *et al.* 1969) makes it extremely unlikely, however, that fertility in a male could ever be achieved. In females, on the other hand, there seems no obvious theoretical reason why an occasional ovulated egg containing a balanced haploid horse or donkey genome, if fertilized, should not give rise to a successful conception.

One problem remains however. As stated earlier in the paper, from the recorded observations on allegedly fertile she-hybrids, there is a total absence of the type of offspring expected if conception arose out of the fertilization of a hybrid ovum containing its *paternal* chromosome set. This could mean either that paternal chromosome sets are excluded from the ova of female hybrids, perhaps by a drive mechanism operating at meiosis to send them into the polar body (Anderson, 1939) or that pregnancies resulting from such fertilized ova are inviable. In male hybrids, is it possible that the reverse situation obtains, i.e. that *maternal* chromosomes are eliminated from their germ cells? Polar bodies are not formed at meiosis in the male, but expulsion of chromosomes during spermatogenesis might occur, as happens for example in males of some other species

(White, 1973). In this connexion the early observations of Wodsedalek (1916) are of some interest. On page 29 of his lengthy and detailed account of the cytology of meiosis in sectioned spermatocytes of the mule he records his observations concerning the 'isolation and expulsion' of a group of chromosomes (variable in number) from the nucleus at telophase I in some cells, and, from the appearance of the chromosomes, inclines to the view that 'the material expelled from the cells is that which was contributed by the mother of the hybrid'. Now if Wodsedalek's (1916) observations and interpretation of events are correct (and it has to be admitted that confirmation of them is still awaited), it would follow that spermatozoa from hybrid animals would contain only paternal chromosome sets, i.e. donkey chromosomes in the case of mule spermatozoa and horse chromosomes in the case of hinny. Furthermore, spermatozoa from hybrids should thus always be Y-bearing, those of the mule carrying a donkey Y chromosome, those of the hinny a horse Y chromosome.

Only a direct analysis of mule and hinny gametic genomes will, however, put an end to such speculation. A truly well documented case including eye-witness of the birth and full karyotyping of mother and offspring is needed finally to end scientific scepticism on the issue of mule fertility.

REFERENCES

- ANDERSON, W. S. (1939). Fertile mare mules. *Journal of Heredity*, **30**, 549-551.
- BENIRSCHKE, K. & SULLIVAN, M. M. (1966). Corpora lutea in proven mules. *Fertility and Sterility* **17**, 24-33.
- BENIRSCHKE, K., LOW, R. J., SULLIVAN, M. M. & CARTER, R. M. (1964). Chromosome study of an alleged fertile mare mule. *Journal of Heredity* **55**, 31-38.
- BIELANSKI, W. (1955). Observations on ovulation processes in she-mules. *Bulletin of the Polish Academy of Sciences, Cl. II (Biological Science Series)* **3**, 243-245.
- BIELANSKI, W. (1972). Clinical observations on sexual behaviour and function of reproductive organs in she-mules. In *Riproduzione Animale & Fecondazione Artificiale*, pp. 45-55. Bologna: Edagricole.
- BIELANSKI, W. & ZAPLETAL, Z. (1968). Ovulation in she-mules; a report of two cases. *Proceedings of the 6th International Congress on Animal Reproduction and Artificial Insemination, Paris* **2**, 1555-1558.
- BRATANOV, K., DIKOV, V. & DOKOV, V. K. (1964). Recherche sur l'infécondité chez l'hybride mâle. *Proceedings of the 5th International Congress on Animal Reproduction and Artificial Insemination, Trento* **3**, 560.
- CHANDLEY, A. C., JONES, R. C., DOTT, H. M., ALLEN, W. R. & SHORT, R. V. (1974). Meiosis in interspecific equine hybrids. I. The male mule (*Equus asinus* × *E. caballus*) and hinny (*E. caballus* × *E. asinus*). *Cytogenetics and Cell Genetics* **13**, 330-341.
- EWART, J. C. (1899). *The Penyuik Experiments*. London: A. and C. Black.
- EWART, J. C. (1910). Are mules fertile? *Nature* **85**, 106.
- GATES, W. H. (1926). Japanese Waltzing Mouse: Its origin, heredity and relation to the genetic characters of other varieties of mice. *Publications of the Carnegie Institution* **337**, 83-138.
- GRAY, A. P. (1954). In *Mammalian Hybrids*. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks., England.
- HSU, T. C. & BENIRSCHKE, K. (1969). In *An Atlas of Mammalian chromosomes*. Berlin: Springer.
- LLOYD-JONES, O. (1916). Mules that breed. *Journal of Heredity* **7**, 494-502.
- MAKINO, S. (1955). Notes on the cytological features of male sterility in the mule. *Experientia* **11**, 224-226.
- MICHIE, D. (1953). Affinity: A new genetic phenomenon in the house mouse. Evidence from distant crosses. *Nature* **171**, 26-27.
- MICHIE, D. (1955). Affinity. *Proceedings of the Royal Society. B* **144**, 241-259.

- MIKLOS, G. L. G. (1974). Sex-chromosome pairing and male fertility. *Cytogenetics and Cell Genetics* **13**, 558–577.
- NISHIKAWA, Y. & SUGIE, T. (1952). Studies on reproductive ability of mules. 1. On functions of ovaries. *Bulletin of the National Institute of Agricultural Sciences, Tokyo* **3**, 61–68.
- SAVORY, T. H. (1970). The mule. *Scientific American* **223**, 102–109.
- SMITH, H. H. (1939). A fertile mule from Arizona. *Journal of Heredity* **30**, 548.
- TAYLOR, M. J. & SHORT, R. V. (1973). Development of the germ cells in the ovary of the mule and hinny. *Journal of Reproduction and Fertility* **32**, 441–445.
- TRUJILLO, J. M., OHNO, S., JARDINE, J. H. & ATKINS, N. B. (1969). Spermatogenesis in a male hinny: Histological and cytological studies. *Journal of Heredity* **60**, 79–84.
- WALLACE, M. (1953). Affinity: A new genetic phenomenon in the house mouse. Evidence from within laboratory stocks. *Nature* **171**, 27–28.
- WALLACE, M. & GUNN, R. E. (1965). Affinity in cotton. *Heredity* **20**, 305–308.
- WHITE, M. J. D. (1973). In *Animal Cytology and Evolution*. Cambridge University Press.
- WODSEDALEK, J. E. (1916). Causes of sterility in the mule. *Biological Bulletin* **30**, 1–56.